Supplemental 1

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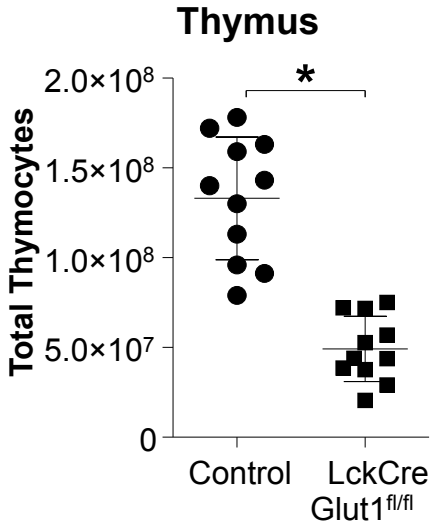

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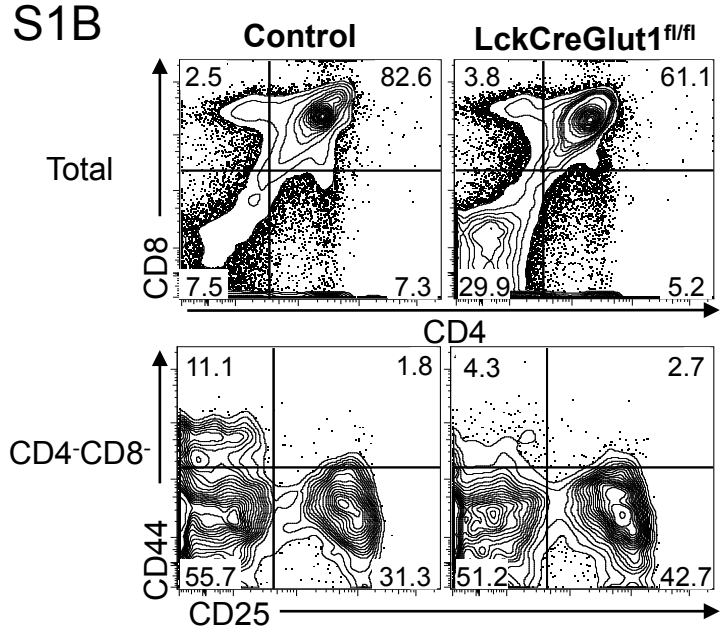

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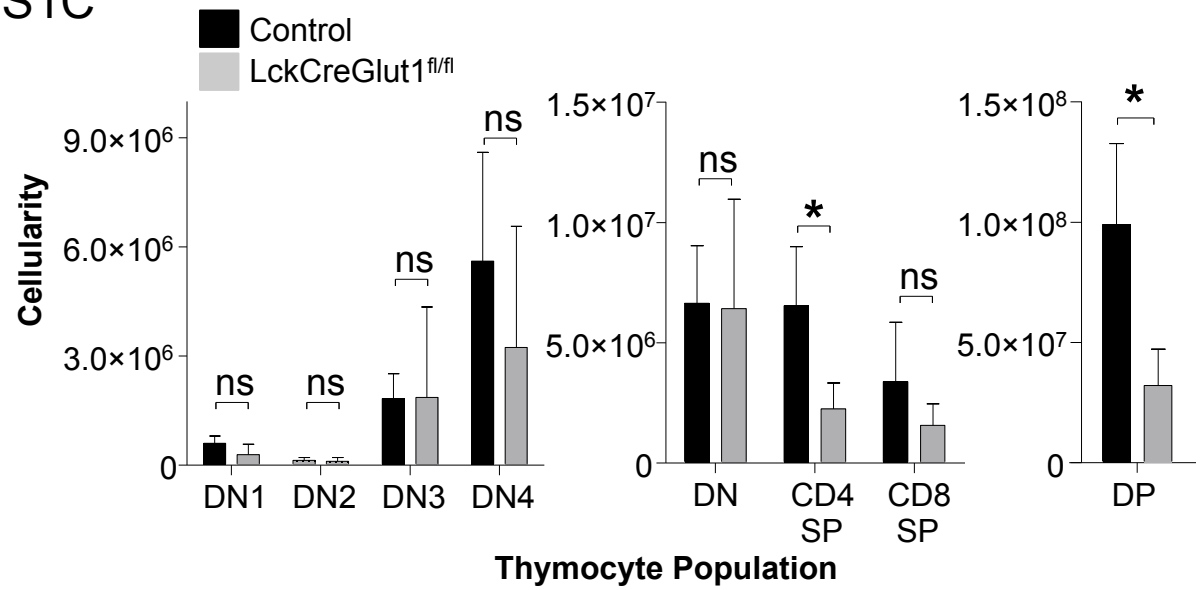

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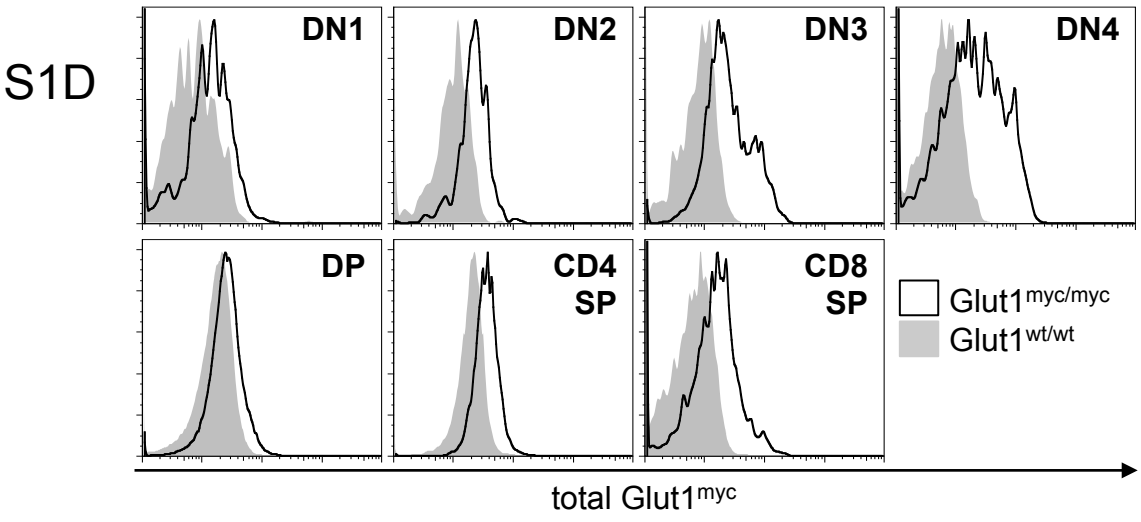

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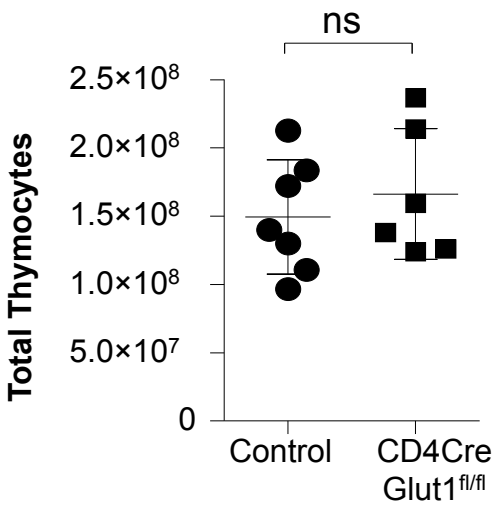

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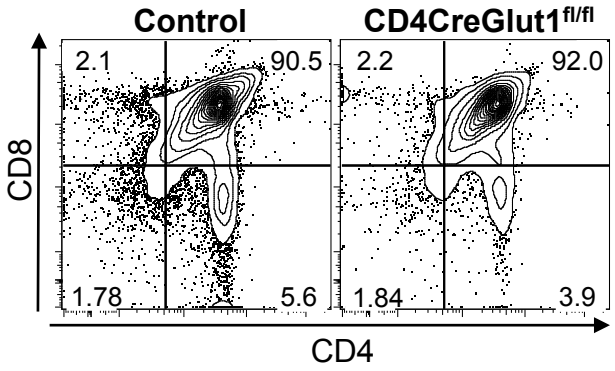

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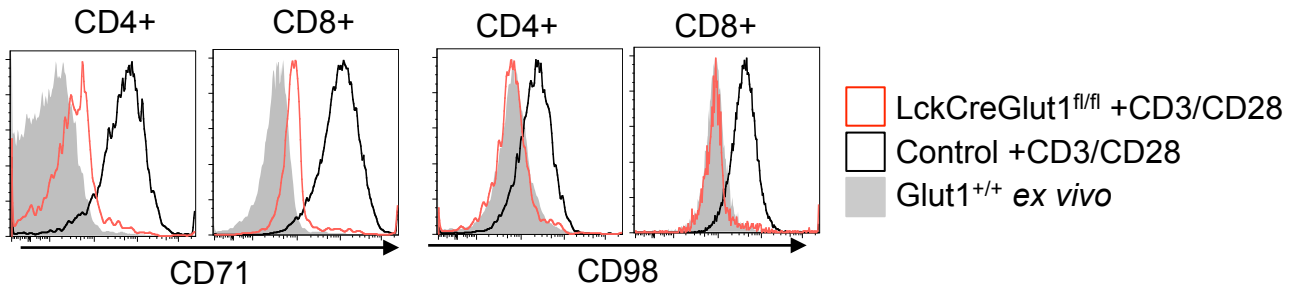

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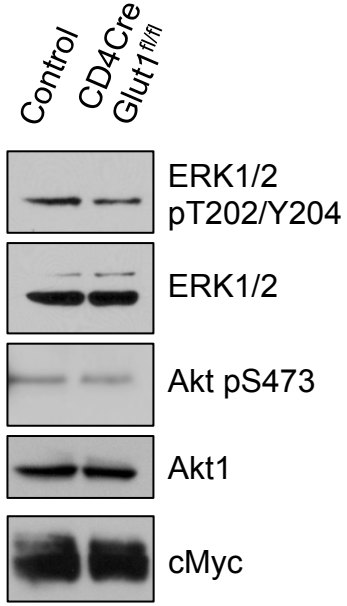

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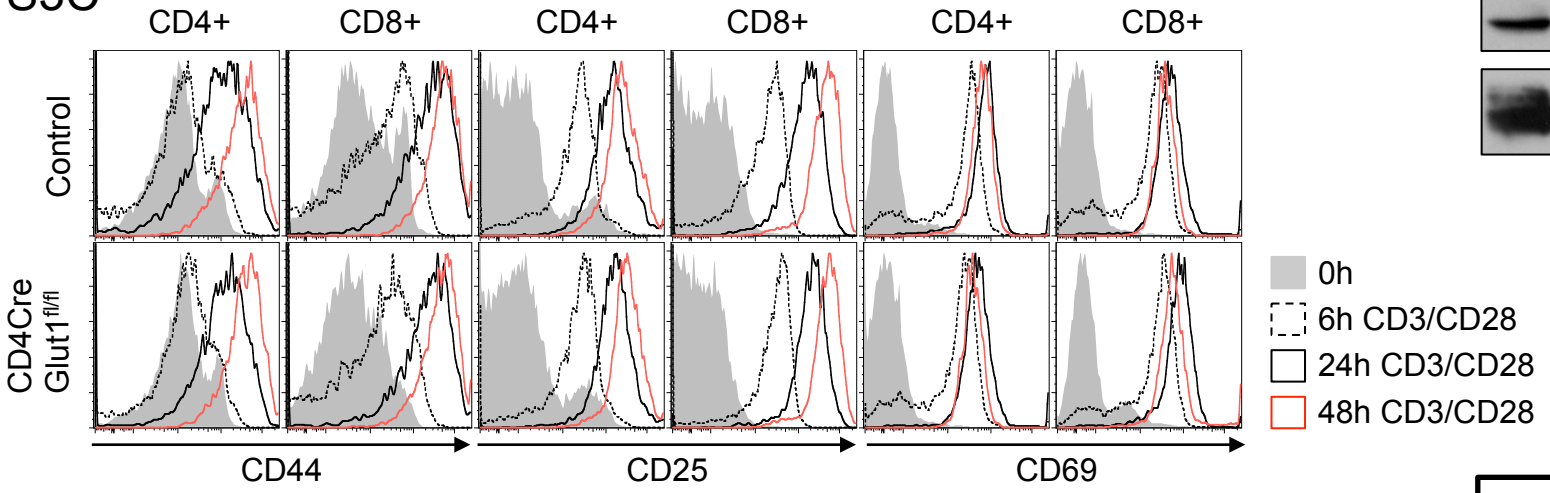

Supplemental 3

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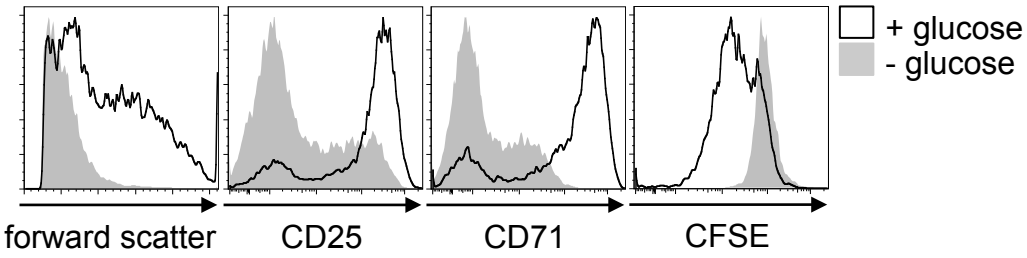

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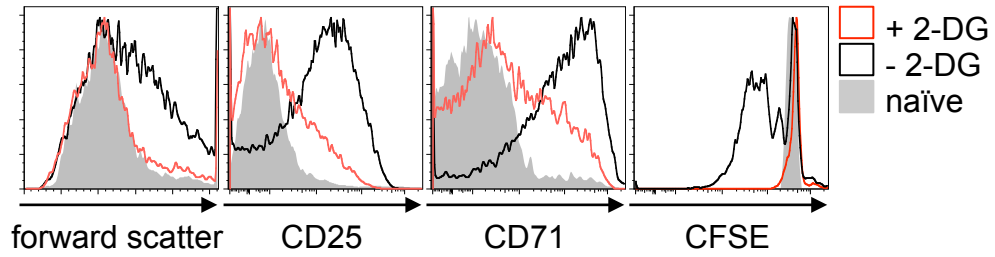

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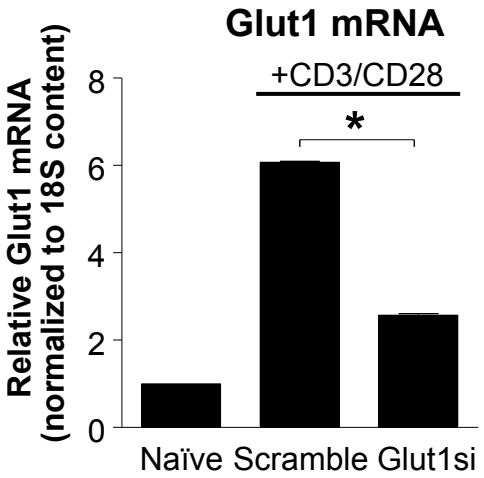

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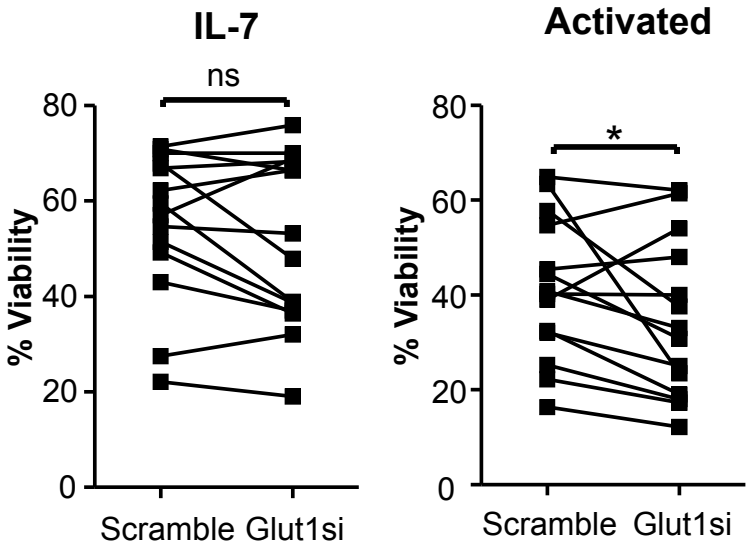
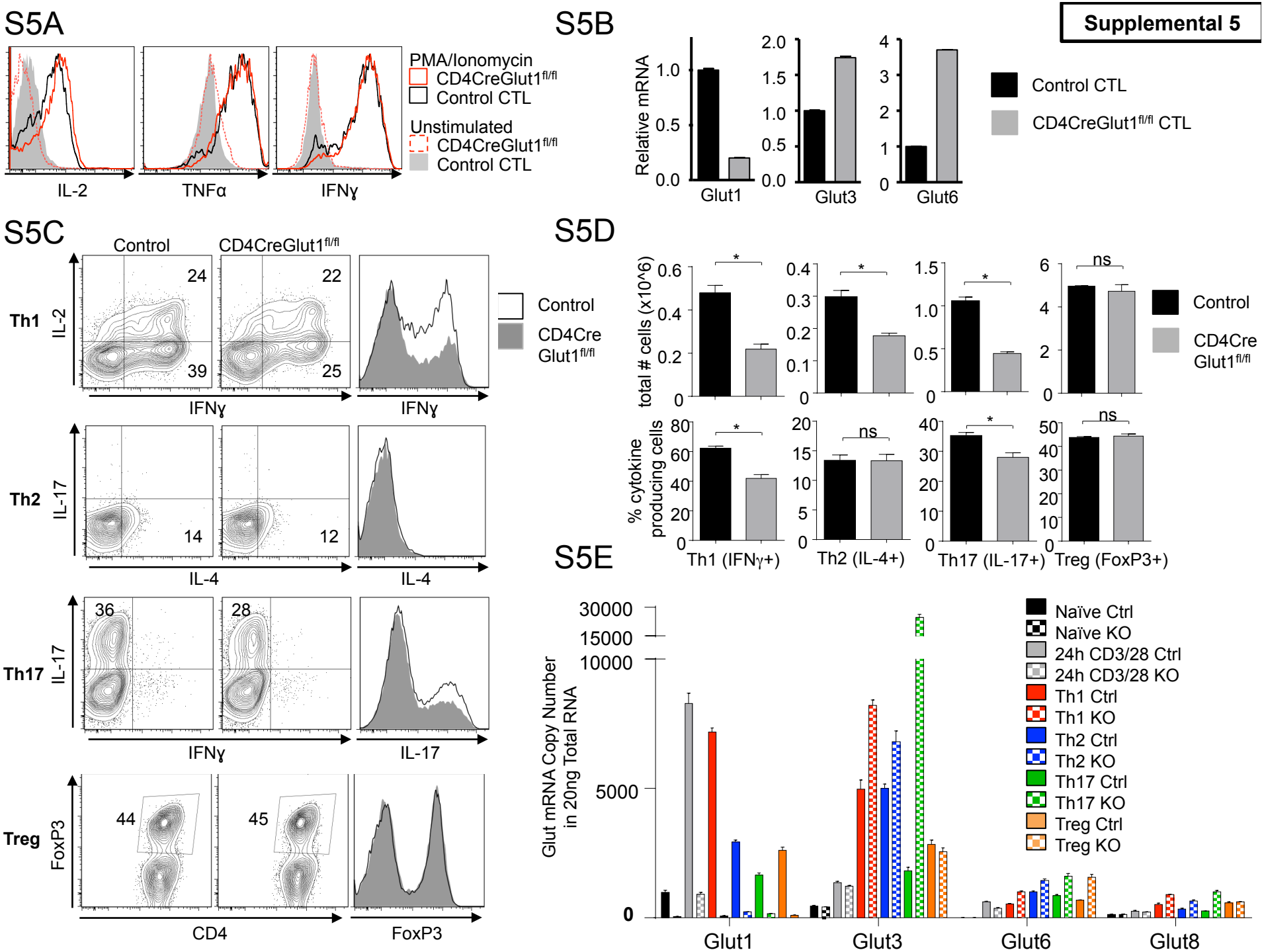


Supplemental 4

Supplemental 5



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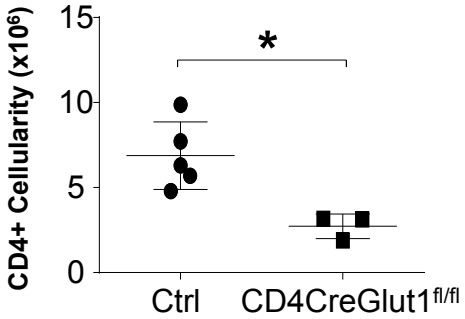

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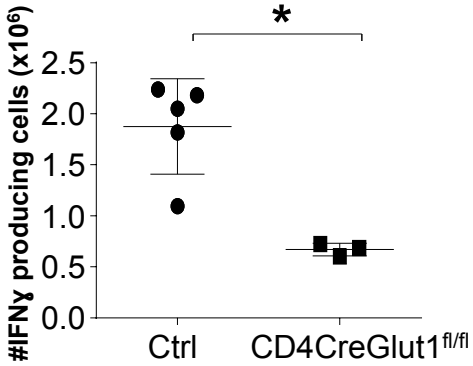

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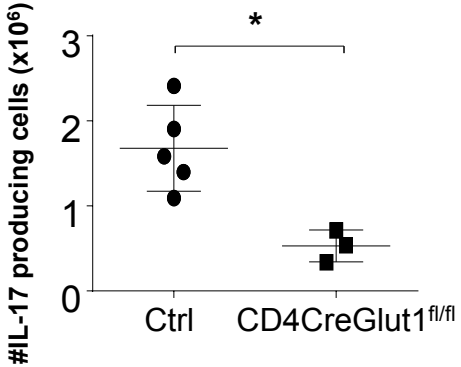

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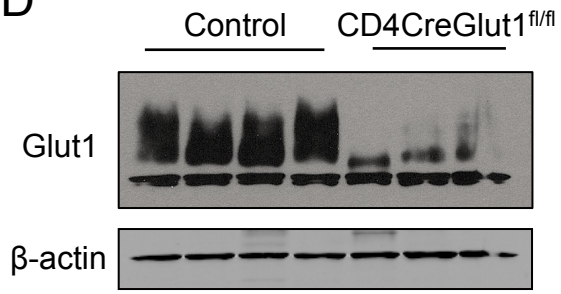
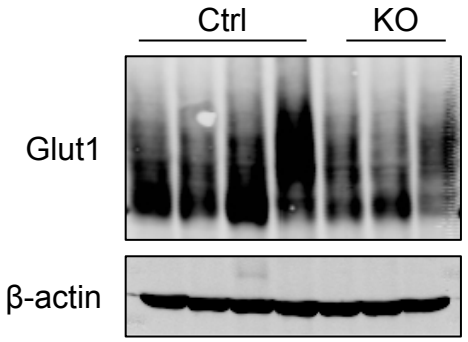


Supplemental 6

S7A



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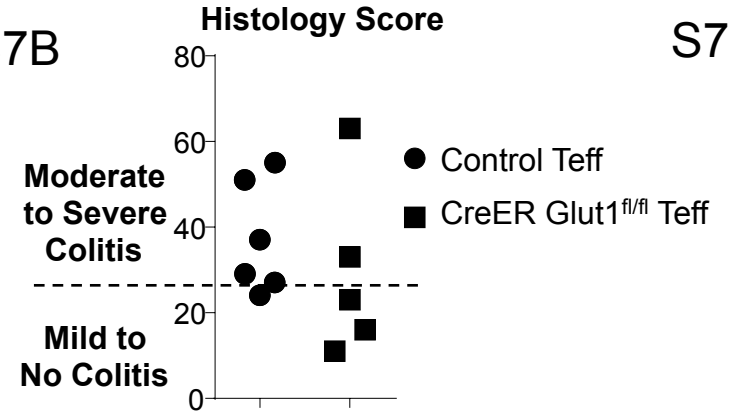

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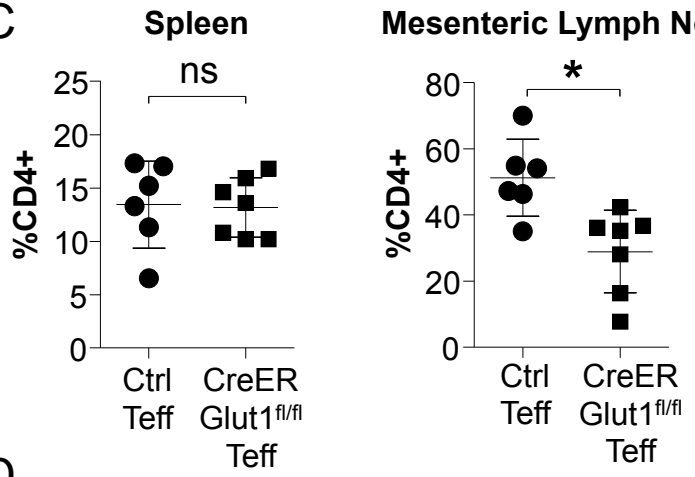

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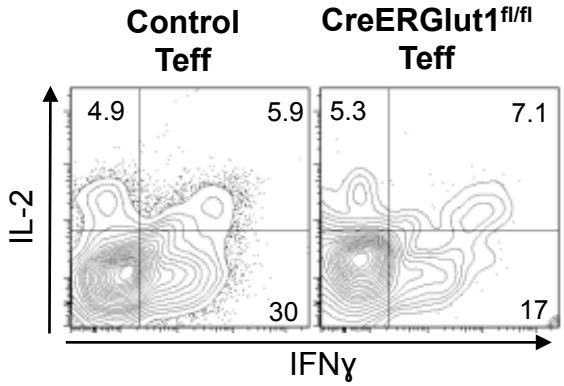

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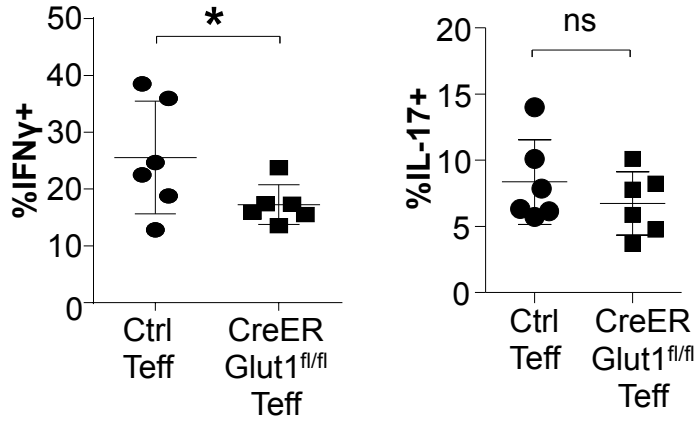

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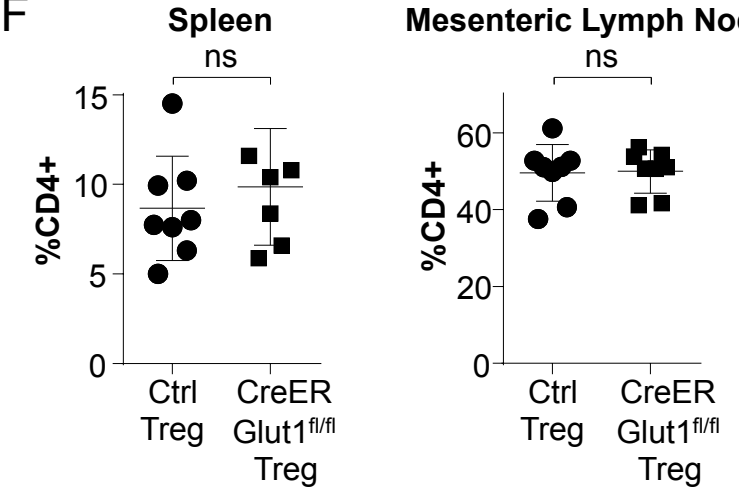

Supplemental 7

SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 1. Glut1 is required to support normal thymocyte development. (**A**) Total thymocyte numbers in control (*Glut1*$^{fl/+}$, *Glut1*$^{+/+}$) and *LckCreGlut1*$^{fl/fl}$ mice. (**B**) Representative flow cytometry of total (upper panels) and CD4$^-$ CD8$^-$ (lower panels) thymocytes from control (*Glut1*$^{fl/+}$) and *LckCreGlut1*$^{fl/fl}$ mice. (**C**) Numbers of control (*Glut1*$^{fl/+}$, *Glut1*$^{+/+}$) and *LckCreGlut1*$^{fl/fl}$ thymocyte subpopulations. (DN1: CD44$^+$CD25$^-$; DN2: CD44$^+$CD25$^+$; DN3: CD44$^-$CD25$^+$; DN4: CD44$^-$CD25$^-$; DP: CD4$^+$CD8$^+$; CD4+ SP: CD4$^+$CD8$^-$; and CD8+ SP: CD4$^-$CD8$^+$). (**D**) Thymi from Glut1$^{myc/myc}$ epitope tag knock-in mice and wild type staining controls were examined by flow cytometry for total Myc tag expression in the indicated thymic Thy1.2+ subsets. (all DN populations: CD4$^-$CD8$^-$; DN1: CD44$^+$CD25$^-$; DN2: CD44$^+$CD25$^+$, DN3: CD44$^-$CD25$^+$; DN4: CD44$^-$CD25$^-$; DP: CD4$^+$CD8$^+$; CD4+ SP: CD4$^+$CD8$^-$; and CD8+ SP: CD4$^-$CD8$^+$). Data shown are mean ± SD from n=11 for **A** and **B**, n=8 for **C** or (**D**) representative of 3 independent experiments.

Figure S2, related to Figure 2. *CD4CreGlut1*$^{fl/fl}$ *mice have normal thymocyte populations*

(**A**) Total thymocyte numbers in control (*Glut1*$^{fl/+}$, *Glut1*$^{fl/fl}$ and *Glut1*$^{+/+}$) and *CD4CreGlut1*$^{fl/fl}$ mice. (**B**) Representative flow cytometry plots of CD8 versus CD4 in control and *CD4CreGlut1*$^{fl/fl}$ mice. Data shown are (**A**) mean ± SD or (**B**) representative of n=6 independent experiments.

Figure S3, related to Figure 3. Glut1 and activation marker expression in LckCreGlut1$^{fl/fl}$ and CD4CreGlut1$^{fl/fl}$ T cells. (**A**) Control (*Glut1$^{fl/fl}$*) and *LckCreGlut1$^{fl/fl}$* T cells were stimulated for 48h and then stained for CD4, CD8, CD71 and CD98 expression. Expression levels are compared to those on wild type C57/B6 T cells directly *ex vivo.* (**B**) Control (*Glut1$^{fl/fl}$*) and *CD4CreGlut1$^{fl/fl}$* T cells were stimulated with anti-CD3 and anti-CD28 for 16h and then analyzed by immunoblot. (**C**) Control (*Glut1$^{fl/fl}$*) and *CD4CreGlut1$^{fl/fl}$* T cells were stimulated for the indicated time and then stained for CD4, CD8, CD44, CD25 and CD69 expression. Data are representative of a minimum of 2 or more independent experiments.

Figure S4, related to Figure 5. Impact of glycolysis inhibition on human T cell activation.

(**A**) Human peripheral blood T cells were activated with anti-CD3 and anti-CD28 for 48h in glucose-free RPMI supplemented with 10% dialyzed serum ±10mM glucose. Data show cell size as assessed by flow cytometry for forward light scatter, expression of CD25, CD71, and CFSE dilution in cells labeled with CFSE prior to stimulation. (**B**) Peripheral blood T cells from healthy donors were either rested (10ng/ml IL-7; naive) or stimulated for 48h with anti-CD3 and anti-CD28 ±10mM 2-deoxyglucose (2-DG). Data show cell size as assessed by flow cytometry for forward light scatter, expression of CD25, CD71, and CFSE dilution in cells labeled with CFSE prior to culture. (**C**, **D**) Peripheral blood T cells from healthy donors were transfected with scramble siRNA or

Glut1si then either cultured in 10ng/ml IL-7 or stimulated with anti-CD3 and anti-CD28 for 48h. Data show (**C**) Glut1 mRNA content in naïve, stimulated scramble and stimulated Glut1si cells, (**D**) viability of cells after 48h stimulation as determined by propidium iodide exclusion. Lines indicate paired samples from the same donor. Data are representative of three (**A**) or two (**B, C**) independent experiments, or (**D**) show aggregated data from 14 independent experiments. Mean ± SD are shown (**C**).

Figure S5, related to Figure 6. Effector CTL and CD4 Teff and Treg subsets produce cytokines and show some compensation for Glut1 loss with other Glut transporters. (**A, B**) cytotoxic T lymphocytes (CTL) were generated using CD8 T cells from control (*Glut1*$^{fl/+}$) and *CD4CreGlut1*$^{fl/fl}$ mice. (**A**) Cytokine generation by PMA/Ionomycin re-stimulated CTL was determined by flow cytometry. (**B**) CTL were generated using CD8 T cells from control (*Glut1*$^{fl/+}$) and *CD4CreGlut1*$^{fl/fl}$ mice. Data show the relative expression of Glut1, Glut3, and Glut6 mRNA in each CTL population. (**C, D**) CD4 T cells from control (*Glut1*$^{fl/+}$, *Glut1*$^{fl/fl}$) and *CD4CreGlut1*$^{fl/fl}$ mice were polarized *in vitro* for 3 days to generate Th1, Th2, Th17 or Treg cells. (**C**) Cytokine production (Th1, IFNγ+; Th2, IL-4+; Th17, IL-17+) or transcription factor staining (Treg, FoxP3+) was measured. (**D**) Total cell numbers and the percentage of cytokine producing or FoxP3 expressing cells in each polarized culture were measured by flow cytometry. (**E**) Glut family mRNA copy number in naïve, stimulated or differentiated CD4 T cells from control (*Glut1*$^{fl/+}$, *Glut1*$^{fl/fl}$) and *CD4CreGlut1*$^{fl/fl}$ mice. Data shown are (**A-D**) representative of (**A**, **C**) FACS plots or (**B**, **D**) mean ± SD from two independent experiments to show or (**E**) mean ± SD of pooled T cell mRNA from 2 mice.

Figure S6, related to Figure 7. T cell expression of Glut1 is required for T cell expansion and function in colitis

Rag1$^{-/-}$ mice were injected with naïve effector ($CD4^+CD25^-CD45RB^{hi}$) control (*Glut1$^{fl/fl}$*) or *CD4CreGlut1$^{fl/fl}$* T cells (Teff) and colitis was initiated after two weeks by treatment with piroxicam. (**A**) The number of CD4 T cells or (**B, C**) IFNγ or IL-17 producing cells in the spleen was determined using flow cytometry and (**D**) Glut1 expression by immunoblot of CD4 T cells isolated from the spleen and mesenteric lymph nodes of control (*Glut1$^{fl/fl}$*) or *CD4CreGlut1$^{fl/fl}$* T cells 4-5 weeks later. Mean ± SD are shown (**A-C**).

Figure S7, related to Figure 7. T cell expression of Glut1 is selectively required for Teff, but not Treg, expansion and function in colitis

Rag 1$^{-/-}$ mice were reconstituted intraperitoneally via injection of naïve effector ($CD4^+CD25^-CD45RB^{hi}$) T cells (Teff) from control (*CreERGlut1$^{+/+}$*, Glut1$^{fl/fl}$) or CreERGlut1$^{fl/fl}$ mice. (**A)** CD4 T cells were isolated and Glut1 expression was examined by immunoblot. (**B**) H&E stained colon sections were examined by microscopy and assessed for colitis severity; a score above 22 indicates moderate to severe colitis. (**C**) Percentage of CD4 T cells or (**D,E**) IFNγ or IL-17-producing cells present in the mesenteric lymph nodes were examined by flow cytometry. (**F**) Rag 1$^{-/-}$ mice were co-injected with wild type naïve T cells together with either control (*CreERGlut1$^{+/+}$*, *Glut1$^{fl/fl}$*) or *CreERGlut1$^{fl/fl}$* Treg cells ($CD4^+CD25^+CD45RB^{lo}$). After two weeks mice were treated

with piroxicam to trigger colitis and tamoxifen to activate Cre. The percentage of CD4 T cells present in the spleen and mesenteric lymph nodes of diseased animals was determined using flow cytometry 4-5 weeks later. Data (**A**-**E**) are representative of three independent experiments. Mean ± SD are shown (**C, E**, **F**).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Human T cell Isolation, siRNA, Stimulation, and Culture

T cells were isolated from healthy, anonymized donors by density gradient centrifugation and magnetic bead negative selection (≥90% purity; Miltenyi Biotec). T cells were cultured in RPMI 1640 (MediaTech) supplemented with 10% fetal bovine serum (FBS) (Gemini Bio-Products), L-glutamine (Gibco), penicillin-streptomycin (Gibco), and 50μM β-mercaptoethanol (β-ME) (Sigma-Aldrich). Where indicated, cells were supplemented with 10mM 2-deoxyglucose (2DG; Sigma-Aldrich) or washed and cultured in glucose-free RPMI (MediaTech) with 10% dialyzed FBS (Gemini Bio-Products), 10mM L-glutamine (Gibco), penicillin-streptomycin (Gibco), 50μM β-ME (Sigma-Aldrich), supplemented as indicated with D-glucose (Sigma-Aldrich). Human siRNA pools (Dharmacon: Glut1, *Slc2a1*, M-007509; or scrambled pool, D-001810-10) were transiently transfected by nucleofection (Amaxa; Human T cell Nucleofector kit program U14; Lonza) and cells were rested 4-6h before stimulation. Where indicated cells were activated on plates coated with 10μg/ml anti-CD3 (clone UCHT1) and 10μg/ml anti-CD28 (eBioscience), stimulated in the presence of 20ng/ml IL-2 (Novartis), or rested in 10ng/ml IL-7 (eBioscience).

Mice

Mice expressing myc-epitope tagged Glut1 (*Glut1*^{myc}) or carrying floxed Glut1 (*Slc2a1*^{fl}) alleles were described previously (Michalek et al., 2011a; Young et al., 2011). *Glut1*^{fl} animals were crossed to mice expressing Cre recombinase under the control of the

p56Lck, *CD4* or *Ubi* (CreER^{T2}) promoters and OT-II TCR (Ovalbumin-specific) transgenics (Jackson Laboratory). BALB/c mice were purchased from the Jackson Laboratory. Mice were bred and maintained under barrier conditions under Duke University Medical Center Institutional Animal Care and Use Committee (IACUC)-approved protocols. Experiments were performed using sex-matched mice between 6 and 12 weeks of age.

Murine T cell Isolation, Stimulation and Culture

Murine total, CD8 or CD4 T cells were isolated by magnetic bead negative selection (≥90% purity; Miltenyi Biotec) and cultured in RPMI 1640 (MediaTech) supplemented with 10% FBS (Gemini BioProducts), L-glutamine (Gibco), penicillin-streptomycin (Gibco), and 50μM β-ME (Sigma-Aldrich). Where stated, cells were cultured in 10ng/ml IL-7 (eBioscience) or activated by stimulation on plates coated with 5μg/ml anti-CD3 (clone 2C11) and 5μg/ml anti-CD28 (eBioscience). Where indicated, cells were stimulated in the presence of 10μM LY294002 (Sigma-Aldrich), 20nM rapamycin (Cell Signaling), or 1μM PP242 (Sigma-Aldrich).

Extracellular Flux Analysis

Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured with an XF24 extracellular flux analyzer (Seahorse Bioscience) using Cell-Tak (BDBiosciences) in unbuffered RPMI (Sigma-Aldrich) supplemented with 10mM D-glucose (Sigma-Aldrich) and 10mM L-glutamine, as described (Wu et al., 2007) and normalized to cell number.

Flow Cytometry, Proliferation and Viability Measurements

Murine T cells were labeled in 2% FBS PBS with: anti-mouse CD4-allophycocyanin (APC), -fluorescein isothiocyanate (FITC), -eFluor 450; CD25-phycoerythrin (PE); CD8 PE-Cy5.5; CD44-Violet Blue; CD69-PE; CD71-APC; CD98-PE; or Thy1.2-FITC (eBioscience). Glut1^{myc} was stained with mouse anti-myc (Millipore) followed by rat anti-mouse IgG-PE (eBioscience). To measure intracellular cytokines, cells were rested or stimulated with PMA (5hr; 50ng/ml; Sigma-Aldrich) and ionomycin (750ng/ml; Calbiochem) in the presence of GolgiStop (IL-4, IL-17) or GolgiPlug (IFNγ, IL-17, IL-2, TNFα), permeabilized (Cytofix/Cytoperm Plus; BDBiosciences), and stained with IFNγ-APC, IL-2-PE, IL-17-PE, TNFα-APC, or IL-4-APC (eBioscience). FoxP3-PE was used with Regulatory T cell staining kit #1 (eBioscience). Human T cells were labeled in 2% FBS PBS with anti-human CD4-Violet Blue, CD8-FITC, CD71-APC, CD25-PE, or CD98-PE (Miltenyi Biotec). Some cells were fixed in 1% paraformaldehyde, permeabilized with methanol and labeled with CD4-Violet Blue, CD8-FITC, and rabbit anti phospho-S6 (Ser235/236; Cell Signaling, 2211) or rabbit anti-Glut1 (Abcam ab115730) followed by rat anti-rabbit IgG-E or APC (eBioscience). For intracellular granzyme B staining cells were fixed in 1% paraformaldehyde, permeabilized with methanol, and then stained with anti-granzyme B-PE (eBioscience). Cell proliferation was assayed by flow cytometry of carboxyfluorescein succinimidyl ester (5μM CFSE; Molecular Probes) or CellTrace Violet (2.5μM CTV; Invitrogen) labeled cells and viability was determined by propidium iodide exclusion (1μg/ml PI; Invitrogen). To assay bromodeoxyuridine (BrdU) incorporation cells were cultured with 10μM BrdU (Sigma-Aldrich) for 8h, fixed in

ethanol and then stained with Alexa-Fluor 647 anti-BrdU (Invitrogen B3514). Cells were labeled with 10μg/ml PI for cell cycle analysis and analyzed as described (Darzynkiewicz and Juan, 2001). Data were acquired on a MacsQuant cytometer (Miltenyi Biotec) and analyzed with FlowJo software (TreeStar).

T cell differentiation

To generate CD4 T cell subsets *in vitro*, naïve $CD4^+CD25^-$ T cells were polarized as described(Michalek et al., 2011a). Briefly, $CD4^+CD25^-$ T cells were cultured 1:5 on irradiated splenic feeder cells (300Gy) with 2.5μg/mL of anti-CD3 (eBioscience) with: Th1, 10ng/mL IL-12 (R&D Systems), 10ug/mL anti-IL-4 (eBioscience), 1μg/mL anti-IFNγ (eBioscience); Th2, 1000U/mL recombinant IL-4 (R&D Systems), 10μg/mL anti-IL-12 (eBioscience), 10μg/mL anti-IFNγ; Th17, 20ng/mL IL-6 (R&D Systems), 2.5ng/mL TGFβ (R&D Systems), 10μg/mL anti-IFNγ; Treg, 3ng/mL TGFβ. Cells were re-plated on day 3 and cultured in 20ng/ml IL-2 alone for 2 additional days and examined by intracellular flow cytometry for T-bet (Th1), GATA3 (Th2), RORγt (Th17) or FoxP3 (Treg) (all PE conjugated from eBioscience). To generate $CD8^+$ cytotoxic T lymphocytes (CTL), $CD8^+$ T cells were stimulated for 48h with 5μg/ml anti-CD3 and 5μg/ml anti-CD28 (eBioscience) in the presence of 20ng/ml IL-2. Cells were then cultured with 20ng/ml IL-2 alone for a further 72h.

Glucose transporter family expression

RNA was isolated using RNAeasy Plus Mini kit (Qiagen). Glut family absolute copy number was determined as described (Rudolph et al., 2011). Briefly, total RNA was

converted to cDNA (Verso cDNA synthesis kit with blended oligo dT; random hexamer primers, Thermofisher) for qPCR (Absolute Fast qPCR Mix-lox Rox; ThermoFisher) with the 7500 fast thermocycler (Applied Biosystems). Taqman Gene Expression Assays (Applied Biosystems) and amplicon sizes are given in Supplementary Table 1. Ct values were fitted to regression curves for each glucose transporter target to quantify transcript copy number. The following primer sets from Applied Biosystems: Glut1, Mm01192270 m1; Glut2, Mm00446224 m1; Glut3, Mm00441483 m1; Glut4, Mm01245507 g1; Glut5, Mm00600311 m1; Glut6, Mm00554217 m1; Glut7, Mm01260620 m1; Glut8, Mm00444634 m1; Glut9, Mm00455116 m1; Glut10, Mm00453716 m1; Glut12, Mm00619244 m1; Glut13, Mm01306489 m1; SGLT1 (Slc5a1), Mm00451203 m1.

Semi-quantitative real time PCR

RNA was extracted (RNeasy RNA purification minikit; Qiagen) and reverse transcribed (iScript cDNA synthesis kit; Biorad) for real-time PCR (iQSYBR Green detection chemistry and iCycler; Biorad). mRNA levels were normalized to 18S RNA or β-2 microglobin using the $\Delta\Delta C_t$ method. Primers used: 18S (GTAACCCGTTGAACCCCATT, CCATCCAATCGGTAGTAGCG), human Glut1 (CACTCCTGTTACTTACCTAA, CACTTACTTCTGTCTCACT), murine Glut1 (AGCCCTGCTACAGTGTAT, AGGTCTCGGGTCACATC), murine Glut3 (TAAACCAGCTGGGCATCGTTGTTG, AATGATGGTTAAGCCAAGGAGCCC), murine Glut6 (TTGGTGCTGTGAGGCT, TGGCACAAACTGGACGTA), murine β-2 microglobin (ACCGGCCTGTATGCTATCCAGAAA, GGTGAATTCAGTGTGAGCCAGGAT).

Immunoblotting

Immunoblotting was performed as described previously (Jacobs et al., 2008) for phospho-AMPK (Thr172, Cell Signaling Technology (CST) 2535), AMPKα (CST 2532), phospho-ERK1/2 (Thr202/Tyr204, CST 9101), ERK1/2 (CST 4695), c-myc (CST 9402), phospho-Akt (Ser473, CST 4051), Akt1 (CST 2967), phospho-S6 (Ser235/236, CST 2211), S6 (Cell Signaling, 2217), Glut1 (Abcam ab115730), Glut3 (Millipore AB1344) or β-actin (Sigma A5441), followed by mouse- or rabbit-conjugated horseradish peroxidase (HRP) (CST) for enhanced chemiluminescence (Thermofisher) or fluorescently labeled anti-mouse or rabbit antibodies (LiCor) and imaged (Odyssey; LiCor).

T cell transfer model of colitis

Naïve effector ($CD4^+CD25^-CD45RB^{hi}$) and regulatory ($CD4^+CD25^+CD45RB^{lo}$) T cells were sorted (FACSvantage; BDBioscience). Naïve effector T cells from were injected i.p. into 6-8 week old C57BL/6 $RAG1^{-/-}$ recipients ($4x10^5$ cells/mouse). Treg cells ($2x10^5$ cells/mouse) were co-injected as indicated. Because the mice were *H. pylori* negative, and colitis does not occur spontaneously in this setting, disease was initiated two weeks following T cell injection with 200ppm piroxicam (Sigma-Aldrich) in powdered rodent chow for 5 days to induce gut damage and colitis (Hale et al., 2005). Mice were then injected i.p with 4mg/kg/day of tamoxifen (Sigma-Aldrich) dissolved in corn oil (Sigma-Aldrich) for 4 days to induce $Glut1^{fl}$ deletion and monitored three times weekly. For analysis the colon was divided into 5 segments representing the cecum, and proximal, mid-, distal, and terminal colon/rectum. Tissues were fixed and a board-certified

pathologist blindly scored the severity of colonic inflammation in hematoxylin and eosin-stained sections as described (Hale et al., 2005).

REFERENCES

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